

Contents lists available at [ScienceDirect](http://ScienceDirect)**Acta Haematologica Polonica**journal homepage: [www.elsevier.com/locate/achaem](http://www.elsevier.com/locate/achaem)**Review/ Praca pogładowa****Genetic alterations in B-acute lymphoblastic leukemia****Seyedeh Momeneh Mohammadi<sup>1</sup>, Daryosh Mohammad Nejad<sup>2</sup>,  
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**ABSTRACT**

Considerable advances have been made in treatment of acute lymphoblastic leukemia (ALL) with an overall survival rate of 85% in children, and with a great improvement in adults. Despite this improvements and the accessibility of hematopoietic stem cell transplantation, relapsed ALL remains a leading cause of childhood mortality emphasizing the need of new approaches on therapy. Understanding of the pathobiology and genetic alteration of ALL has been enhanced by developing molecular technologies including microarray analysis and genome sequencing. These studies have helped identifying mutations in key signaling pathways and revolutionized the treatment of ALL by drugs which specifically target the genetic defects of leukemia cells, such as tyrosine kinase inhibitors. In this paper, we review the clinically important Genetic Alterations in ALL.

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**Introduction**

Acute lymphoblastic leukemia (ALL) is the most common cancer in children, with top prevalence in the ages of 2–5 years and the important cause of mortality from hematological cancers in adults with second peak after the age of 50 years [1, 2]. Totally children with B lineage ALL have a favorable clinical outcome in comparison with those suffering from T lineage ALL [3]. Considerable advances have been made in treatment of ALL with an overall survival rate of 85% in children [2]. About adults, the outcome is being

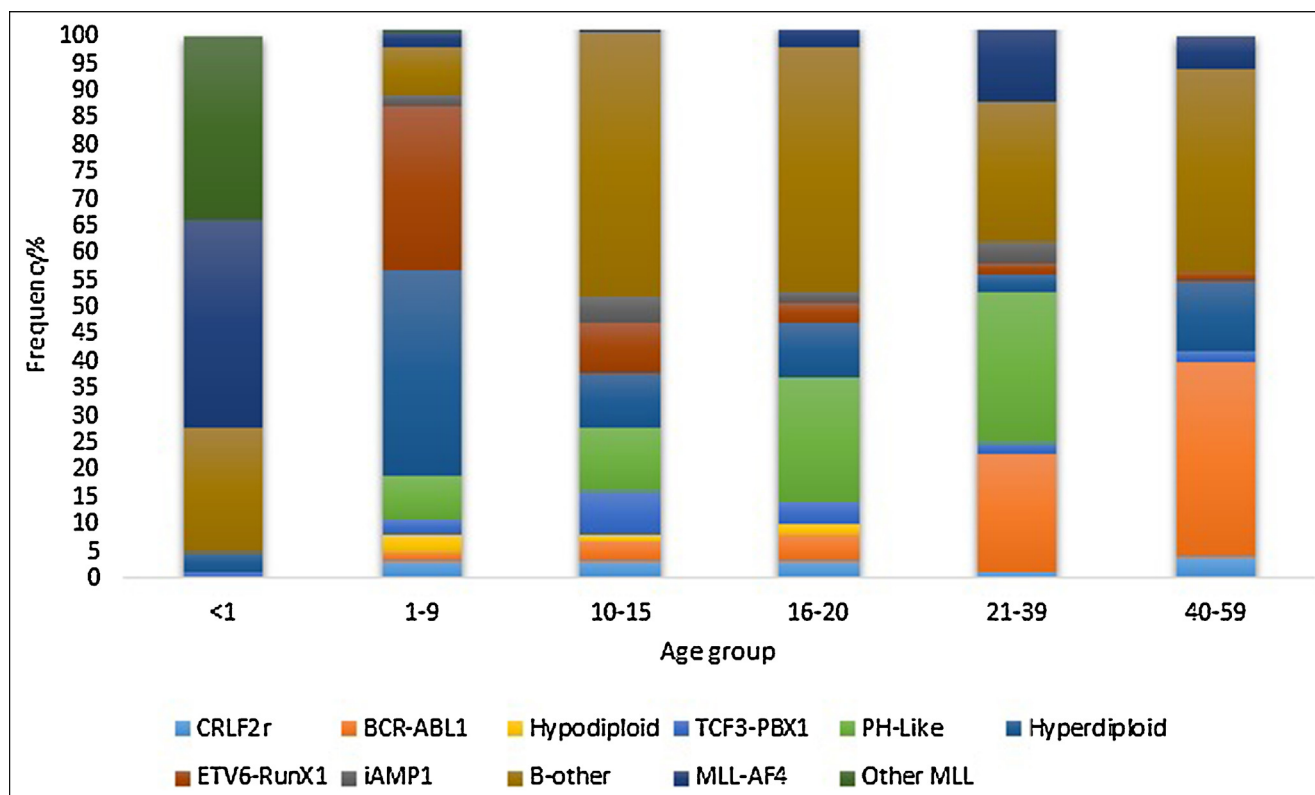
improved but it remains poor compared to children with a long-term survival of only 45% [1]. The reasons for these differences are multifactorial and not fully understood, but with increasing age, the frequency of genetic alterations with favorable outcome decreases and alteration with poor outcome like BCR-ABL are more common [2]. Although, all chromosomal translocations is occurred in any age and in both children and adults but there is a significant difference in the incidence of approximately most subgroups based on age (Fig. 1) [4]. For instance ETV6- Ranx1 and hyperdiploidy dominate in young children, with low incidence of this rearrangement in adults (3%), whereas the incidence of

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**Fig. 1 – Distribution of ALL subtypes according to age groups. The occurrence of ALL. Subtypes varies between age groups. Abbreviations: ALL, acute lymphoblastic leukemia; B-other, B-cell acute lymphoblastic leukemia with other subtypes; CRLF2-r, CRLF-2-rearranged; MLLr, MLL-rearranged; Ph-like, Philadelphia chromosome-like [68, 69]**

BCR-ABL1 Philadelphia translocation increases considerably with age, 3% in children versus 25% in adult [4]. This biological difference highly likely affects outcome and overall survival in adults which is significantly inferior to children.

### Primary genetic subtypes of B-cell precursor ALL

The fundamental mechanisms of leukemia transformation are the same and contain, abnormal expression of proto-oncogenes and chromosomal translocation that finally create fusion genes encoding active kinases and transformed transcription factors. Such abnormalities cause ALL by enforcing in lymphoid progenitor cells an unrestricted

ability for self-renewal, which typically followed by lacking of control on normal proliferation, a block in cell differentiation and almost resistance to normal apoptosis [5]. The gross chromosomal alterations are hallmark of ALL, and the occurrence of each alteration varies with age (Tab. I). The most common rearrangements observed in B-lineage ALL are the t(12;21) (p13;q22) rearrangement resulting in the expression of TEL-AML1 fusion, the t(9;22) (q34;q11.2) “Philadelphia” chromosome resulting in the expression of BCR-ABL1 fusion, the t(1;19) (q23;p13) translocation resulting in the expression of TCF3-PBX1 fusion; and t(4;11) (q21;q23) translocation encoding MLL-AF4 fusion. Identification of these rearrangements is important in diagnosis and risk stratification of patients with ALL. Two main genetic subtypes TEL-AML1 (ETV6-RUNX1) positive and hyperdiploidy

**Table I – Clinically relevant subgroups of ALL**

Chromosomal translocations/gene fusions	Prevalence (%)	Relative prognosis
High hyperdiploidy with more than 50 chromosomes	~30	Excellent
Hypodiploidy with less than 44 chromosomes	2–3	Poor
t(9;22)(q34;q11.2) translocation encoding BCR-ABL1 fusion	2–4	Poor/ improved with imatinib or other tyrosine kinase inhibitor
t(12;21)(p13;q22) translocation encoding ETV6-RUNX1 fusion	15–25	Excellent
t(4;11)(q21;q23) translocation encoding MLL-AF4 fusion	1–2	Poor
t(1;19)(q23;p13) translocation encoding TCF3-PBX1 fusion	2–6	Excellent
Ph-like ALL	10–15	Multiple cytokine receptor and kinase-activating lesions; amenable to tyrosine-kinase inhibitor therapy

with greater than 50 chromosome compromise the most common abnormalities (approximately 50%) in pediatric precursor B-ALL cases. Both of them have favorable outcome and are classified in good risk cytogenetic abnormalities most likely due to sensitivity of TEL-AML1 to L-asparaginase and hyperdiploidy to L-asparaginase and antimetabolites such as 6-mercaptopurine and methotrexate [3, 6, 7]. While in adults, the most common genetic abnormalities is Philadelphia chromosome translocation t (9;22) BCR-ABL1, which encodes the activated BCR-ABL1 tyrosine kinase. It is a reciprocal translocation between chromosome 9 and 22 which fuses the ABL1 oncogene on chromosome 9, to a breakpoint cluster region (BCR) from chromosome 22 and is related to a high risk of relapse. Other abnormalities with high risk of relapse and poor outcome are rearrangement of mixed-lineage leukemia genes (MLL) in both groups.

The protein with histone methyl transferase activity, which is encoded by MLL gene is essential for hematopoietic regulation of HOX-A and MEIS1 gene expression. The most common gene rearrangements include t(4;11) encoding MLL-AFF1, t(9;11) encoding MLL-MLLT3, t(11;19) encoding MLL-ENL

and t(10;11) encoding MLL-MLLT10, between mentioned rearrangements the t(4;11) translocation is the most common with 50% incidence. In general, MLL rearrangements are related with adverse outcome highly likely due to cellular drug resistance [8]. Of note, "BCR-ABL1-like" or "Ph.-like" ALL, is a newly described subtype of high risk ALL that is related with poor outcome, and increased in frequency with age (which will be described in more details in the following section) [9].

### Submicroscopic genetic aberration in ALL

Primary oncogenic events such as chromosomal rearrangements are not sufficient to cause leukemia by themselves and secondary mutations should accompany this chromosomal translocation. Advances in cytogenetic utilizing array based technology have revealed additional submicroscopic abnormalities in genes that are involved in normal hematopoiesis, apoptosis and tumor suppression (Tab. II).

**Table II – recently identified genetic alteration in ALL**

Gene	Alteration	Frequency	Pathophysiologic and clinical consequences of alteration	References
CRLF2	Rearrangement (as IGH@-CRLF2 or P2RY8-CRLF2)	5–15% pediatric and adult B-ALL, and >50% Down syndrome (DS) ALL, and 50% of Ph-like ALL	Concomitant JAK1/2 mutations in >50% of cases; associated with IKZF1 alteration and poor outcome, particularly in non-DS-ALL.	[10, 11, 13]
IKZF1	Focal deletions or sequence mutations	15% of all pediatric patients with B-ALL: 70–80% of BCR-ABL1+ patients with ALL and 30% OF high-risk BCR-ABL1 like B-ALL	Associated with poor outcome in both BCR-ABL1-positive and negative ALL cases	[40, 44]
PAX5	Deletions, translocations, sequence mutations	31% of B-ALL	Transcription factor required for B-lymphoid development; mutations impair DNA binding and transcriptional activation but not associated with poor outcomes	[44, 45]
JAK1/2	Pseudokinase and kinase domain mutations	Up to 10% of high-risk BCR ABL1-like B-ALL; 18–35% of DS ALL	Concomitant CRLF2 rearrangement, associated with poor outcome; may be responsive to JAK inhibitors	[18, 52]
Kinase rearrangements and mutation	Rearrangements of ABL1, ABL2, CSF1R, EPOR, PDGFRB;	10% childhood B-ALL, up to 30% adult ALL; associated with Ph-like gene expression profile	Activation of kinase signaling pathways and associated with increased risk of relapse	
IL7R	Up to 7% of B and T-ALL	Sequence mutations	In B-ALL is associated with the aberrant expression of CRLF2, results in receptor dimerization with CRLF2 and JAK-STAT activation, may be responsive to JAK inhibitors	[61–64]
NT5C2	Sequence mutations	19% of relapse T cell ALL and 3% of relapse B-precursor ALL	mutants confer resistance to treatment with nucleoside analog therapies	[65]
TP53	Deletions and sequence mutations	Present in 12.4% of relapse B cell ALL and of 6.4% relapse T cell ALL	Associated with disease relapse and poor event-free survival and overall survival.	[66]
CREBBP	Deletions and sequence mutations	19% of relapsed B-ALL	Mutations result in impaired histone acetylation and transcriptional regulation and associated with glucocorticoid resistance	[67]

## Cytokine receptor like factor 2 rearrangements in ALL (CRLF2)

CRLF2 rearrangement has been detected in approximately 5–15% of childhood and adult B-cell precursor and also they are more common in Down syndrome ALL (in 50% of cases) [10–12]. CRLF2 encodes cytokine receptor like factor 2 (also known thymic stromal lymphopoietin receptor/ TSLPR) which forms a heterodimeric receptor for thymic stromal lymphopoietin (TSLP) with interleukin-7 receptor- $\alpha$  (IL7R) [13]. Two alterations have been typically recognized in CRLF2; a chromosomal translocation which juxtaposes CRLF2 to the immunoglobulin heavy chain locus (IGHG) or more commonly a focal deletion upstream of CRLF2 resulting in fusion of CRLF2 gene with G protein-coupled purinergic receptor P2Y8 gene (P2RY8) [14]. While the P2RY8-CRLF2 alteration is more common in younger pediatric ALL, but IGH-CRLF2 is frequently detected in adults with ALL [12, 15]. Both of the rearrangements result in overexpression of CRLF2 on the cell surface of leukemia lymphoblast which can be detected by flowcytometry [12]. Less commonly alteration of CRLF2 is a point mutation at codon 232 which replace a phenylalanine with a cysteine. Various groups have tried to describe prognostic value of CRLF2 alteration and also its association with treatment outcome in patients with ALL. It has been shown that Patients with a high CRLF2 expression had a high rates of relapse ( $31\% \pm 8\%$  vs  $11\% \pm 1\%$ ,  $P = 0.006$ ) and a poor event free survival (EFS) ( $61\% \pm 8\%$  vs  $83\% \pm 2\%$ ,  $P = 0.003$ ) compared to Patients with a low CRLF2 expression [16]. In a cohort study, it was shown that ALL Patients with overexpression of CRLF2 have a very poor outcome [17]. Another cohort study of pediatric ALL reported that there is a high occurrence of CRLF2 genomic lesions in DS-ALL (in more than 50% of cases) [11]. Additionally, shRNA knockdown of CRLF2 in B-ALL cell lines only partly inhibited cell growth [15], which suggest that CRLF2 overexpression alone is not sufficient to transform cells and other cooperating mutations and covariates may be involved. The best described of this covariates that accompany CRLF2 alteration are involving janus kinase1 and januse kinase 2 mutations, IKZF1 mutations and DS-ALL. Half of patients with CRLF2 overexpression also harbor JAK mutations (JAK 1 and JAK 2) [17]. Mutation in the pseudokinase domain of JAK2 at R683 is the most common [18]. CRLF2 rearrangement together with JAK2 mutants resulted in constitutive JAK-STAT activation and cytokine-independent cell growth [11]. It was revealed that in 41 CRLF2-rearranged DS-ALL patients, 34% of them also had JAK2 mutations [11]. In line, in study of 26 high-risk pediatric B-ALL patients with CRLF2 overexpression, 69% of them had JAK mutations [19]. CRLF2 rearrangements also have revealed to be associated with gain of function mutations of IL7R in patients with ALL [20, 21]. The mutant IL-7R proteins forms a functional receptor with CRLF2 for TSLP and resulting in cytokine-independent growth of progenitor lymphoid Cells [20]. Importantly, CRLF2 rearrangements are surrogates of poor outcome and can be used in risk stratification and targeting therapy.

## IKZF1 gene deletions

During recent years, between recurring genetic alterations which cooperate in leukemogenesis, the lymphoid transcription factor gene IKZF1 has been found to have definite prognostic impact in B-ALL [22, 23]. IKZF1, which encodes IKAROS protein member of family of zinc finger, is required for the development of all lymphoid lineages [24] and has been established as one of the most clinically relevant tumor suppressors in high-risk acute lymphoblastic leukemia. IKZF1 deletions usually result in the expression of dominant-negative IKAROS variants (e.g., IK6) that are characterized by loss of N-terminal zinc fingers (which mediate DNA binding) and result in the loss of the tumor suppressor function attributed to wild-type IKZF1 [25, 26]. The incidence of IKZF1 deletions in children with Philadelphia chromosome-positive (Ph+) ALL is approximately 70%, whereas its incidence in children with Philadelphia chromosome-negative (Ph-) ALL is 10–15%, and is related with an increased risk of relapse and decreased overall survival in both groups [24, 27–30].

## Intrachromosomal amplifications of chromosome 21 (iAMP21) in ALL

Intrachromosomal amplification of chromosome 21 (iAMP21) is an uncommon high-risk Chromosomal abnormality that occur in approximately 2–5% of pediatric patients with B-cell precursor ALL [31–33]. Fluorescence in situ hybridization (FISH), using RUNX1, provides the only reliable detection method (five or more RUNX1 signals per cell) [34]. Patients with iAMP21 are older (median 9 years vs 5 years) with a low white cell count (median 3.9 vs 12.4) compared to children without this abnormality [35]. In a study of, 1630 ALL patients were treated on the UK MRC ALL97 protocol, iAMP21 was recognized as an independent predictor of poor EFS (29% vs 78%) and OS (71% vs 87%) at 5 years [35]. The results of two cohorts of patients with B-cell precursor ALL and iAMP amplification (2%) treated on ALL97 or UKALL2003 showed that iAMP21 patients with ALL benefitted from receiving more intensive therapy in UKALL2003 (event-free survival (29% vs 78%), relapse (70% vs 16%) and overall survival rates (67% vs 89%) at 5 years) [36].

## BCR-ABL1-like or “Ph-like” ALL

Ph-like ALL, is a newly described subtype of ALL which exhibit a gene expression profile similar to that of Ph+ ALL but lacks BCR-ABL1 fusion gene [37–39]. Deletions or mutations of IKZF1 are hallmark of both BCR-ABL1-positive and Ph-like ALL which strikingly is associated with treatment failure and disease relapse in both [7, 30, 38, 40]. The incidence of Ph-like ALL increases with age, from 10–15% of childhood B-ALL to over 25% of ALL in young adults (Fig. 1). The prognosis of BCR-ABL1-like ALL is poor. In the COG AALL0232 study, the EFS of the BCR ABL1-like cases was significantly inferior to that of the non BCR-ABL1 like cases

**Table III – Kinase rearrangements and therapeutic targets in Ph-like ALL [9]**

Kinase	Tyrosine kinase inhibitor
ABL1	Dasatinib
ABL2	Dasatinib
CSF1R	Dasatinib
PDGFRB	Dasatinib
CRLF 2	JAK2 inhibitor
JAK2	JAK2 inhibitor
EPOR	JAK2 inhibitor
DGKH	Unknown
IL2RB	JAK1/JAK3 inhibitor
NTRK3	Crizotinib
PTK2B	FAK inhibitor
TSLP	JAK2 inhibitor
TYK2	TYK2 inhibitor

(62.6%  $\pm$  6.9% vs. 85.8%  $\pm$  2.0%) [41]. Rearrangements and sequence mutations in several classes of cytokine receptors and tyrosine kinases are a hallmark of Ph-like ALL [42]. Kinase-activating alterations were identified in 91% of patients with Ph-like ALL; rearrangements involving ABL1, ABL2, CRLF2, CSF1R, EPOR, JAK2, NTRK3, PDGFRB, PTK2B, TSLP, or TYK2 that are responsive to treatment with currently available TKIs (Tab. III) [9].

## PAX5

The PAX5 gene, belongs to the paired box (PAX) gene family of transcription factors, crucial for B lymphoid cell commitment [43]. Somatic alterations of the PAX5 are a hallmark of B-ALL and occurred in over one – third of this patients [44, 45]. These alterations are heterozygous and comprise focal deletions, translocations, or point mutations that disrupt PAX5 DNA-binding or transcriptional regulatory functions [46]. Several studies showed that PAX5 abnormalities were not associated with an unfavorable prognosis and are not associated to outcome [44, 46]. Also in study of 89 patients with ALL, deletions of PAX5 was observed in 29 patients which had no prognostic significance in ALL [47].

## Janus kinase 1 and 2 mutations (JAK1 or JAK2) and JAK-STAT pathway in ALL

Janus kinase (JAK) is a family of intracellular, non-receptor tyrosine kinases that transduce cytokine-mediated signals via the JAK-STAT pathway [48, 49]. The JAK family has four members: JAK1, JAK2, JAK3, and TYK2 that functioning as signal transducers to control cellular proliferation, survival, and differentiation [49]. The incidence of JAK mutation in ALL has been reported to be about 18 [50, 51] to 35% in DS-ALL [52] and about 10% in high risk BCR-ABL1 negative ALL and have been associated with poor outcome [18]. Notably, between other family members, JAK2 mutations are more common (at or near JAK2 R683) in B-progenitor ALL [18, 50]. Importantly, JAK mutations were shown to be associated with CRLF2 rearrangements, it has been shown

that 70% of CRLF2-rearranged cases also harbor JAK mutations [19]. Over expression of CRLF2 with JAK2 mutants resulted in cytokine-independent cell growth and constitutive activation of JAK-STAT signaling pathway, demonstrating that these two genetic lesions together contribute to leukemogenesis in B-progenitor ALL [19]. The signal transducers and activators of transcription (STAT) family is one of the best characterized downstream that is activated by JAK signaling. It contains a number of latent transcription factors that, when phosphorylated by the JAKs, drive the expression of genes involved in proliferation, apoptosis, migration, differentiation [53–55]. Disrupted or dysregulated JAK-STAT pathway result in immune deficiency syndromes and cancers [56]. Gain-of-function mutations in IL7R is one of the other mutations that activate the JAK-STAT signaling pathway and have been identified in patients with ALL. Activating mutations in IL7R are commonly found in T and B cell ALL and are frequently placed in the transmembrane domain [20, 57, 58]. IL7R is essential for normal lymphoid development [59]. IL7R heterodimerizes either with interleukin 2 receptor subunit gamma (IL2RG) to form a receptor to IL-7 or with CRLF2 to form a receptor to TSLP [60, 61]. Activating mutations in IL7R lead to dimerization and constitutive activation of the IL-7 receptor and resulting in cytokine independent activation of the downstream signaling pathways including JAK-STAT signaling pathway [57].

## Conclusions

Obviously genome sequencing has revolutionized our knowledge of the genomic basis of ALL. These techniques helped us to identify new clinically important ALL subtypes and leukaemogenic alterations, which have led to a better risk stratification and will lead to improvement of patient-directed or individualized therapy for every patient. However, more comprehensively sequenced ALL genome is required to fully understand all somatic genetic alteration of ALL that incorporate to treatment failure and disease relapse.

## Authors' contributions/ Wkład autorów

SMM – study design, data collection and interpretation, manuscript preparation, literature search. HNC, DMN – data interpretation, funds collection.

## Conflict of interest/ Konflikt interesu

None declared.

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## Ethics/ Etyka

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; Uniform Requirements for manuscripts submitted to Biomedical journals.

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